PB-215 096

FACTORS INFLUENCING THE CONDENSATION OF 4-AMINOANTIPYRINE WITH PHENOLS

Samuel D. Faust, et al

Rutgers - The State University
New Brunswick, New Jersey

1964

DISTRIBUTED BY:

NTIS

National Technical Information Service
U. S. DEPARTMENT OF COMMERCE
5285 Port Royal Road, Springfield Va. 22151

PB 215 096

"Factors Influencing the Condensation of 4-Aminoantipyrine with Phenols"

by

Samuel D. Faust and Frederick C. Lorentz

Associate Professor and Research Assistant
Department of Environmental Science
Rutgers, The State University
New Brunswick, N.J.

Presented at the
11th Ontario Industrial Waste Conference
June 21 - 24, 1964
Bigwin Inn, Ontario

Reproduced by
NATIONAL TECHNICAL
INFORMATION SERVICE
U S Department of Commerce
Springfield VA 22151

"Factors Influencing the Condensation of 4-Aminoantipyrine with Phenols"*

by

Samuel D. Faust and Frederick C. Lorentz
Associate Professor and Research Assistant
Department of Environmental Science
Rutgers, the State University
New Brunswick, N.J.

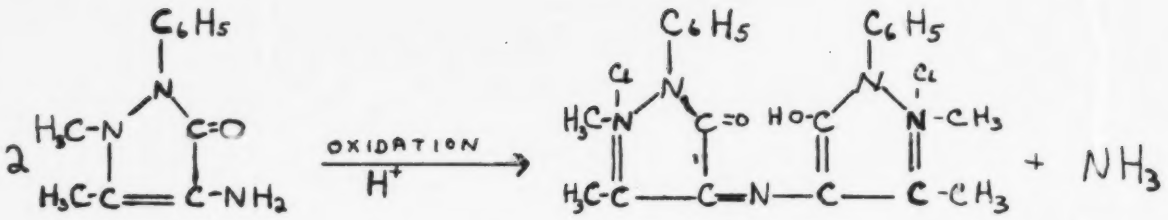
Phenolic compounds condense with 4-Aminoantipyrine and by subsequent oxidation under alkaline conditions produces a color the intensity of which is proportional to the phenol concentration. These condensation and oxidation reactions have been applied to the detection and determination of phenols in water and waste waters. Herein are reported the physical and chemical factors influencing these reactions from a critical review of the literature and from our own research.

Original Work

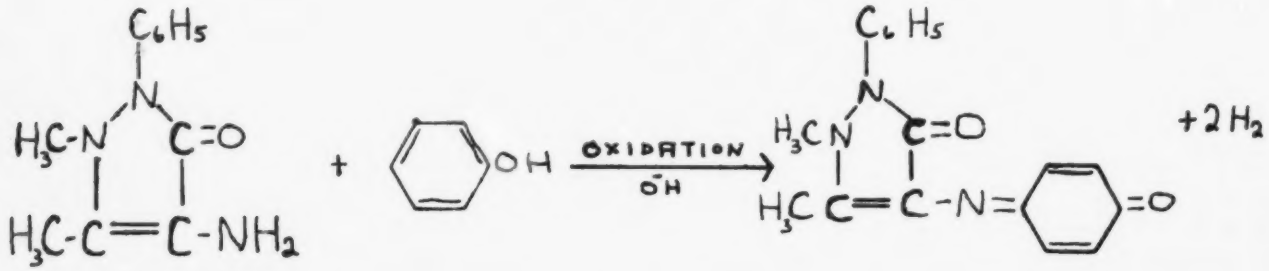
Emerson first reported that two moles of aminoantipyrine are condensed and oxidized to antipyrine red under acid conditions with the elimination of one mole of ammonia (reaction 1, figure 1).(1) This reaction was extended to the oxidation of aminoantipyrine in the presence of various aromatic amines to produce indamine-type dyes. Emerson noted also that phenols gave color reactions but under pH conditions entirely different than the amines.

Subsequently, the alkaline oxidation of phenol in the presence of 4-aminoantipyrine by potassium ferricyanide was investigated by Emerson (reaction 2, figure 1).(2) Phenol was detected in "one part in

*Paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers, the State University of New Jersey, Department of Environmental Science, New Brunswick, New Jersey.



Condensation and Oxidation of 4-Aminoantipyrine



Condensation and Oxidation of the 4-Aminoantipyrine Phenol Complex

Figure 1.

over eight million, o-cresol in one part in two million, and m-cresol in one part in over six and one-half million." Red to purple colors were produced when the alkalinity was sufficiently high enough to prevent the formation of antipyrine red.

Emerson subsequently investigated the reactivity of a large number of phenols, Tables I and II, from which was concluded:

1. "There must be at least one free phenolic hydroxyl group in the molecule for a positive test.

2. "Substituents in the position para to the hydroxyl group prevent the reaction except as follows: halogen, carboxyl, sulfonic acid, hydroxyl, and methoxyl. These groups are probably expelled.

3. "A nitro group in the ortho position prevents reaction and a nitro group in the meta position inhibits the test but not completely.

4. "Coupling of aminoantipyrine with phenol takes place in the para position rather than in the ortho position."

5. No color reaction occurs when the ortho positions are open and the para position is blocked by alkyl, aryl, ester, nitro, benzoyl, nitroso, or aldehyde groups.

Emerson tried NaOH, $NaHCO_3$, Na_2CO_3, and NH_4OH to control pH in various empirical combinations with potassium ferricyanide. An absolute pH value was not specified other than to recommend that the system be alkaline enough to prevent formation of the interfering antipyrine red color.

In a subsequent study, Emerson and Kelly again used $NaHCO_3$, Na_2CO_3, NH_4OH, and NaOH to determine the most satisfactory base (Table III).

Table I.

Phenols Reacting with Aminoantipyrine - Emerson[a]

Phenol	Reaction[b]	Substitutions on Ring	Position
Phenol	3-S	---	---
o-Cresol	3-S	CH_3	2
M-Cresol	3-S	CH_3	3
3,5-Dimethyl-	3-S	CH_3	3,5
2,5-Dimethyl-	3-S	CH_3	2,5
Thymol	3-S	CH_3,C_3H_7	3,6
Carvacrol	3-S	CH_3,C_3H_7	2,5
o-Phenyl-	3-S	C_6H_5	2
o-Cyclohexyl-	3-S	C_6H_{11}	2
o-Hydroxyacetophenone	3-S	$COCH_3$	2
2,4'-Dihydroxybenzophenone	3-Ins.	COC_6H_5OH	2
3,Methyl, 4-Chloro-	3-S	CH_3,Cl	3,4
o-Chloro-	3-S	Cl	2
p-Chloro-	3-S	Cl	4
2,4-Dichloro-	3-S	Cl,Cl	2,4
p-Bromo-	3-S	Br	4
2,6-Dibromo-	3-S	Br,Br	2,6
2,4,6-Tribromo-	3-S	Br,Br,Br	2,4,6
m-Methoxy-	3-S	OCH_3	3
p-Methoxy-	3-S	OCH_3	4
Guiacol K Sulphonate	3-Ins.	OCH_3,SO_3K	2,6

Table I, Cont'd.

Phenol	Reaction[b]	Substitutions on Ring	Position
o-Phenolsulphonic acid	3-Ins.	SO_3H	2
p-Phenolsulphonic acid	3-S	SO_3H	4
o-Carboxyl-	3-Ins.	COOH	2
m-Carboxyl-	3-Ins.	COOH	3
p-Carboxyl-	3-S	COOH	4
Methyl Salicylate	3-S	$COOCH_3$	2
Salol	3-S	$COOC_5H_5$	2
Salicylsalicylic Acid	3-SSI	$COOC_6H_4$ COOH(o)	2
Salicylanide	3-S	$CONH_2$	2
Salcylaldehyde	3-S	CHO	2
3,Methoxy, 4-Carboxyl-	3-S	OCH_3,COON	2,4
2,6-Dibromo, 6-Carboxyl-	3-S	Br,COOH,Br	2,4,6
o-Hydroxy-	3-Ins.	OH	2
m-Hydroxy-	3-Ins.	OH	3
p-Hydroxy-	1-S	OH	4
3,Hydroxy, 5-Methyl-	3-Ins.	OH, CH_3	3,5
4-Tert. Butylcatechol	1-S	OH, C_3H_7	2,5
3,5-Dihydroxy-	3-Ins.	OH,OH	3,5
2,3-Dihydroxy-	3-Ins.	OH,OH	2,3
m-Nitro-	2-S	NO_2	3
m-Amino-	3-S	NH_2	3

(a) Reference 2.

(b) 3, strong test; 2, moderate test; 1, slight test; S, soluble; Ins, insoluble; SSl, slightly soluble.

Table II

Phenols not Reacting with Aminoantipyrine - Emerson[a]

Compound	Substitutions on Ring	Position
Anisole	OCH_3	1
p-Cresol	CH_3	4
2,4-Dimethyl-	CH_3, CH_3	2,4
2,4-Dimethyl-	CH_3, CH_3	3,4
p-Tert. Amyl-	C_5H_{11}	4
p-Tert. Butyl-	C_4H_9	4
p-Tert. Butyl-o-cresol	CH_3, C_4H_9	2,4
p-Tert. Amyl-o-cresol	CH_3, C_5H_{11}	2,4
4-Phenyl-	C_6H_5	4
p-Cyclohexyl-	C_6H_{11}	4
Tyrosine	CH_2NH_2COOH	4
p-Hydroxyacetophenone	$COCH_3$	4
4,4'-Dihydroxybenzophenone	COC_6H_4OH	4
o-Methoxy-	OCH_3	2
Eugenol	$OCH_3, CH_2CH:CH_2$	2,4
iso-Eugenol	$OCH_3, CH:CHCH_3$	2,4
Methyl p-hydroxybenzoate	$COOCH_3$	4
p-Hydroxybenzaldehyde	CHO	4
Vanillin	OCH_3, CHO	2,4
o-Nitro-	NO_2	2
p-Nitro-	NO_2	4
p-Nitroso-	NO	4
p-Amino-	NH_2	4

(a) Reference 2.

Table III

Effect of Excess Bases on the Condensation Reaction of Phenols with 4-Aminoantipyrine[a]

Base	$NaHCO_3$ -0.17M					Na_2CO_3 -0.08M					NH_4OH -0.17M					NaOH -0.17M				
Drops Used	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Compounds																				
Phenol	3[b]	3	2	2	2	3	3	2	2	2	3	3	3	3	3	3	3	2	1	1
2,4-Dichloro-	5	5	5	5	5	5	5	3	3	2	5	5	3	3	3	2	1	-	-	-
2,4,6-Tribromo-	5	5	5	5	5	5	5	1	1	1	5	5	1	1	1	5	-	-	-	-
o-Nitro-	3	3	3	3	2	3	-	-	-	-	3	-	-	-	-	-	-	-	-	-
m-Nitro-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
m-Carboxyl-	1	2	2	2	2	3	3	3	2	2	3	3	3	3	2	2	1	1	1	1
o-Chloro-	5	5	5	5	5	5	5	5	5	3	5	5	5	5	5	5	2	1	-	-
3,5-Dimethyl-	3	4	4	2	2	3	2	2	2	2	1	3	3	2	2	3	2	2	2	2
p-Carboxyl	2	3	3	2	2	3	2	2	2	2	3	3	3	3	2	2	2	1	1	1

(a) Reference 3

(b) Arbitrary color intensity scale: 5 indicates a precipitate was formed, 4, a very intense color, 1, a very weak test.

In general, color formation is dependent upon amount of base, type of base, and type of phenol. The bases, when used in excess, can be arranged in a series of increasing inhibition: $NaHCO_3$ NH_4OH Na_2CO_3 NaOH. Emerson suggested, therefore, that $NaHCO_3$ be used as the alkaline agent. Also, the data in Table III indicate that: (a) different phenols have different sensitivities of reaction with 4-aminoantipyrine, (b) pH or ionic strength affects color intensity, (c) some phenols exhibit a pH effect on maximum color formation as evidenced by 3,5-dimethyl phenol and p-carboxyl phenol, and (d) phenols that have groups expelled from the para position are inhibitied more by excess base.

Wave Length of Maximum Absorbance

Table IV shows the wave lengths of maximum absorbance for several phenols as determined by several investigators[4,5,6,7,8] and from our own research. These values ranged from 495 to 550 mu and generally exhibited broad spectra as exemplified by 2,4-dichlorophenol in Figure 2.[4] pH value and type of buffer appear to influence wave length of maximum absorbance for a given phenol.

Effect of pH on Absorbance

Since the original data of Emerson suggested that pH affects color intensity and that the pH should be high enough to prevent development of the antipyrine red color, several analytical methods were developed at a pH value of 10.0. Gottlieb and Marsh, using a sodium carbonate buffer, effected the reaction at pH 10.4-10.6.[5] Ettinger, et al., suggest a pH range of 9.8-10.2 controlled by a 6N NH_4OH

solution,[6] and Standard Methods recommends an NH_4OH-NH_4Cl buffer at pH 10.0 $\pm$ 0.2.[9]

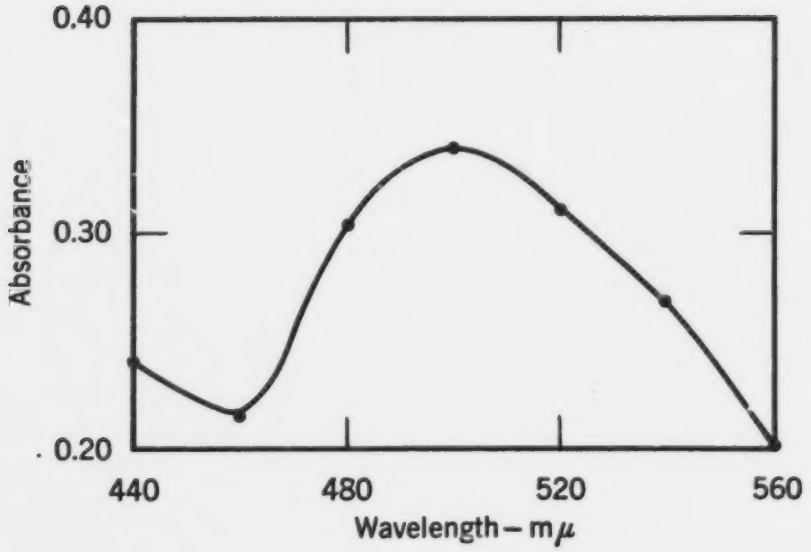
Subsequently, an investigation revealed that at the pH value of 10.0 $\pm$ 0.2, 2,4-dichlorophenol produced very little color, whereas at pH 7.9 $\pm$ 0.1 maximum intensity was developed. Since these results were contrary to those of Gottlieb and Marsh[5] who reported that 2,4-dichlorophenol was determined at pH 10.4 with a Na_2CO_3 buffer, their procedure was repeated: the initial pH of the reaction system was 10.4 but after the 4-aminoantipyrine and $K_3Fe(CN)_6$ reagent were added, the pH fell to 7.9. These results agree with Dannis[10] who also observed the effect of reagent addition on final pH value in an NH_4OH-NH_4Cl buffer system. Consequently, the effect of three aqueous buffers on pH stability of the reaction medium was investigated (Table V). The NH_4OH-PO_4 system provided the greatest stability.

That pH value does affect absorbance readings of phenol-4-aminoantipyrine color complexes is seen in Table VI. In general, as pH was increased from 8.0 to 10.5, absorbance values decreased, the magnitude of which varied with each phenol. For example, the absorbance for 2,4-dichlorophenol decreased from 0.229 to 0.022, whereas for phenol the decrease was from 0.222 to 0.174. Substitution on the phenol ring also apparently affects the reactivity with 4-aminoantipyrine at the higher pH values. This can be seen by comparing the absorbance readings of the tri-substituted phenols with those of the mono- and di-substituted. These results are in general agreement with those of Emerson (Table III).

Table V

Effect of Different Buffers on pH Stability of the Reaction Medium

Ammonium Hydroxide-Phosphate		Ammonium Hydroxide-Ammonium Chloride		Ammonium Hydroxide	
Before pH	After pH	Before pH	After pH	Before pH	After pH
7.6	7.6	8.0	7.8	9.5	8.8
8.0	8.0	8.5	8.3	9.6	9.1
8.5	8.45	9.0	8.8	9.8	9.3
9.0	8.95	9.5	9.3	10.0	9.7
9.5	9.45	10.0	9.8	10.2	9.9
10.0	9.95			10.7	10.4



Absorbance Curve for the 2,4-Dichlorophenol - 4-Aminoantipyrine Complex

Figure 2.

Table VI

Effect of pH Value on Absorbance of Several Phenol-4-Aminoantipyrine Color Complexes

Phenol[a]	pH Value[b]					
	8.0	8.5	9.0	9.5	10.0	10.5
	Absorbance[c]					
Reagent Blank	0.022	0.013	0.009	0.013	0.009	0.004
Phenol	0.222	0.208	0.187	0.131	0.194	0.174
Phenol[d]	0.360	0.360	0.360	0.360	0.360	0.340
p-Methoxy-	0.149	0.137	0.125	0.114	0.125	0.131
p-Bromo-	0.119	0.114	0.065	0.046	0.046	0.046
2,4-Dichloro-	0.229	0.125	0.081	0.051	0.041	0.022
2,4,5-Trichloro-	0.222	0.131	0.071	0.036	0.022	0.013
2,4,6-Trichloro-	0.143	0.081	0.032	0.018	0.009	0.004
4-Chloro-3,5-Dimethyl-	0.131	0.103	0.081	0.065	0.055	0.051

(a) 50 ug

(b) NH_4OH-PO_4 buffer systems

(c) Distilled Water blank, 500 mu, 20° ± 0.5°C., 2.40 cm cells

(d) From ref. 10., 500 ug, 520 mu, NH_4OH-NH_4Cl buffer

Type of buffer is another factor influencing color intensity. Different transmittance values are given for 2,4-dichlorophenol at a given pH value with three different buffers, (Figure 3). The NH_4OH buffer gave the highest color intensity at a given pH value but did not provide stability against pH drop as noted in Table V.

LaCoste, et al., also noted the effect of pH and buffer type on color intensity that was ascribed to variations in ionic strength.[7] Their data are summarized in Table VII from which absorbance values: (a) vary inversely with ionic strength at any pH value as noted by the NH_4OH-NH_4Cl systems, (b) differ with type of buffer, and (c) decrease as pH value increases. LaCoste suggested that variations in color intensity due to ionic strength is a specific ion effect; namely, the ammonium ion which is demonstrated by comparison of the absorbance data from the NaOH-HCl systems with the 1.0M NH_4OH-NH_4Cl and NH_4OH-NH_4Ac buffers. LaCoste chose the NaOH-H_3BO_3 system because "constant color intensity was recorded in the pH range of 8.2 to 10.0 and color intensity is essentially constant at pH 9.2 with ionic strengths of 0.02, 0.2, and 1.0M boric acid." These data and conclusions should be examined, however, with criticism because no mention was made of the effect of reagent addition on pH stability of the various buffers. The variations in absorbance readings, Table VII, may be due to variations in the final pH of the reaction mixtures.

Ochynski compared color intensity development in borate, phosphate, and carbonate buffers.[8] A portion of these data is seen in Table VIII from which Ochynski concluded: "maximum colour formation

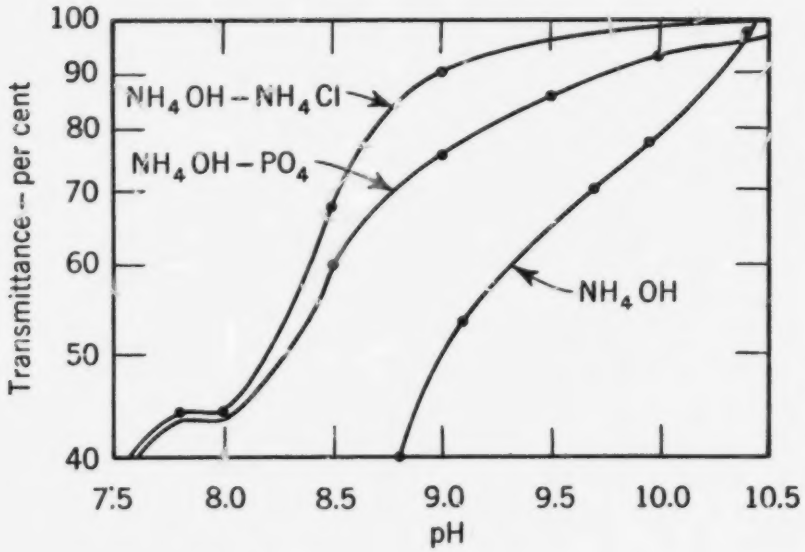
Table VII

Effect of pH and Ionic Strength on Absorbance[a]

Absorbance Readings[b]

	NH_4OH-NH_4Cl				NH_4OH-NH_4Ac	NaOH-HCl		H_3CO_3-NaOH
pH	0.1M	0.5M	1.0M	1.5M	1.0M	0.1M	1.0M	0.4M
8.0	--	--	--	--	0.160	0.470[c]	--	0.420[c]
8.2	0.410	--	--	--	--	--	--	--
9.0	--	--	--	--	0.220	--	--	0.420
9.5	0.410	0.300	0.230	0.100	--	0.450	0.460	0.420
10.0	--	0.330	0.220	0.120	0.250	--	0.450	0.420
10.5	0.440	0.350	0.280	0.140	--	0.400	0.400	0.350
11.0	--	--	--	--	--	0.280	0.320	0.280
12.0	--	--	--	--	--	0.050	0.040	0.030

(a) Reference 7.
(b) Phenol Conc. = 3.3 mg/l.
(c) Phenol Conc. = 3.0 mg/l.



Effect of Type of Buffer on Color Intensity of the 2,4-Dichlorophenol Color Complex

Figure 3.

Table VIII

Light-Absorption Values in Presence of Borate, Phosphate, and Carbonate Solutions[a]

pH	Absorptiometer-Drum Units	
	0.1M NaOH-H_3BO_3	
	[b]	[c]
9.0	0.12	0.23
9.6	0.12	0.23
10.0	0.11	0.22
10.6	0.12	0.23
	0.1M NaOH-KH_2PO_4	
7.7	0.08	0.22
9.2	0.13	0.24
10.7	0.13	0.25
11.0	0.10	0.20
11.5	0.08	0.15
	0.1M Na_3CO_3	
10.5	0.11	0.21

(a) Reference 8.
(b) Phenol Conc. = 2.0 mg/l.
(c) Phenol Conc. = 4.0 mg/l.

for a given concentration of phenol occurs at a pH between 9 and 10.7 and that the chemical composition of the buffer solution has no effect on the light absorption." These conclusions are somewhat in variance with LaCoste,[7] and Faust and Aly[4] but were made from only three buffer systems in which NaOH or Na^+ was the predominant base.

Effect of Type of Phenol

The earlier work of Emerson suggested that type of phenol and type of substitution in the o, m, and p positions affects the degree of reactivity with 4-aminoantipyrine (Tables I, II, and III). Since these data were qualitative, there was no indication of the molecular and analytical sensitivities of these phenols. Table IX shows that analytical and molecular sensitivities vary directly with absorptivity value. In general, these data are in agreement with Emerson, with the exception of 4-methyl-, 3,4-dimethyl-, and 4-phenyl phenol that exhibit some reactivity but low sensitivities.

Substitution on the phenol ring greatly influences the reaction with 4-aminoantipyrine. In group 1, where the para position is open, reaction sensitivity decreases as the number of substitutions increases. In groups 2, 3, 4, 5, and 6, the type of the para substitution as well as that of the ortho and meta groups influences reactivity. In general, halogen groups enhance reactivity whereas alkyl groups suppress reactivity. Variations in the sensitivity of different phenols were observed also by Dannis[10], Ettinger[6], and Mohler and Jacob[11].

Table IX

Effect of Type of Phenol on Absorptivity Values

Group	Para Substitution	Phenol [a]	Molar Absorptivity	Absorptivity
1	H	3-Chloro-	13,500	105.0
	"	Phenol	9,310	98.9
	"	3-Methoxy-	10,100	81.4
	"	2,6-Dimethyl-	5,210	42.6
	"	3,5-Dimethyl-	3,810	31.2
	"	2-Isopropyl,5-Methyl-	2,810	18.7
2	Cl	2,4-Dichloro-	14,300	97.5
	"	2,4,5-Trichloro-	11,200	56.4
	"	2,4,6-Trichloro-	10,500	52.6
	"	4-Chloro-	6,770	52.6
	"	4-Chloro,3,5-Dimethyl-	4,400	27.9
3	Alkyloxy	4-Methoxy-	7,330	61.6
	"	4-Ethoxy-	5,730	41.6
4	Alkyl	4-Methyl-	54.5	0.504
	"	3,4-Dimethyl-	224	1.84
	"	2-Amino,4-Phenyl-	846	4.5
	"	4-Phenyl-	41.2	0.242
5	Amino	4-Amino,3-Methyl-	972	7.91
	"	4-Amino,2,6-Dibromo-	2,480	9.23
6	Br	4-Bromo-	6,230	36.1

(a) 500 mu, 2.24 cm cells, 20° ± 0.5°C., pH 8.0 ± 0.1, reagent blank.

Effect of Interferences

Interferences with the 4-aminoantipyrine and phenol reaction fall into three general types: (a) red color from the oxidation of 4-aminoantipyrine, (b) inorganic reducing substances, and (c) aromatic amines that also condense with 4-aminoantipyrine. Antipyrine red can be eliminated by keeping the reaction pH value above 7.4 as indicated in Table X.

Inorganic sulfides, such as Na_2S, interfere by reaction with the oxidizing agent as quantitated by Gordon[12] (Table XI). This interference has been eliminated by precipitation with $CuSO_4$[9] or $AgNO_3$[12] and by acid extraction of the sample with petroleum ether.[4]

Ettinger observed that aniline produced a color with 4-aminoantipyrine that decreased in intensity as the pH value was increased from 9.6 to 11.15 (Table XI).[6] Consequently, a pH range of 9.8 to 10.2 was suggested that would minimize this interference since 20 mg/l of aniline are equivalent to 0.1 mg/l phenol in absorbance values. Aromatic amines were eliminated also by an acid petroleum extraction technique.[4] Aniline did not interfere up to 20 mg/l by this procedure,

McFarlane observed interferences from colloidally dispersed or suspended organic matter in paper and pulp mill effluents.[13]

Effect of Temperature

The effect of temperature on absorbance values from various 4-aminoantipyrine-phenol color complexes has been documented well by

Table X.

Effect of pH Value on Antipyrine Red Formation

pH	Color Formed	Time After Which Fading Began (Min.)
5.8	Deep Red	30
6.0	" "	30
6.5	" "	30
7.0	Moderate Red	15
7.2	Slightly Red	5
7.4	No Red	-
7.6	" "	-
7.8	" "	-

Table XI.

Effect of Sulfides[a] and Aniline[b]

Na_2S	Phenol[c]	pH	Aniline
mg/l	mg/l		Absorbance[d]
0	4.0	9.6	0.85
10	3.7	9.8	0.50
20	3.5	9.9	0.45
40	2.4	10.1	0.30
60	0	10.5	0.15
80	0	11.15	0.05

(a) Reference 12.
(b) Reference 6.
(c) 510 mu.
(d) 100 mg/l aniline, 460 mu in $CHCl_3$, 1.0 cm.

Ettinger[6], Ochynski[8], and LaCoste[7]. Ettinger found that a 37°C. temperature gave lower absorbance readings than 20°C. which was attributed to color fading. Ettinger also found a higher absorbance reading for the reagent blank at 37°C. than at 20°C. Ochynski, however, reported temperatures of 25° to 50°C. did not affect the absorbance value of 18 mg/l phenol but that the color faded over 50°C. Likewise, LaCoste observed no appreciable effects on any given phenol concentration over the temperature range of 16° to 36°C.

Effect of Order of Reagent Addition and Reagent Concentration

Ettinger suggested that the following order of reagent addition to the phenol shall be: (a) pH buffer, (b) 4-aminoantipyrine, and (c) oxidizing agent.[6] This indicates that the color reaction occurs in the following sequence: (a) ionization of the phenol, (b) condensation with the phenol, and (c) oxidation to the quinoid structure. That the color producing reagents be added in excess was determined by Ettinger[6] and LaCoste[7].

Effect of Color-Complex Stability

There are mild disagreements relative to the stability of color complex (Table XII). Ettinger's data[6] suggested a slow fading over 120 minutes with phenol, whereas Ochynski's data[8] did not. LaCoste found considerable fading of the MEHQ color complex over 25 minutes and within two temperature ranges.[7]

Table XIII shows the effect of time on stability of the 2,4-dichlorophenol color complex at pH 8.0. Maximum absorbance was

Table XII

Stability of Color-Complexes in Aqueous Solution

Time	Phenol[a]	Phenol[b]	MEHQ[c]	MEHQ[c]
Min.	0/6 mg/l	2.0 mg/l		
		Absorbance Values		
0.0	0.37	----	0.310	0.295
5.0	----	0.11	0.300	0.275
10.0	----	----	0.285	0.255
15.0	0.36	----	-----	-----
20.0	----	----	0.275	0.245
25.0	----	----	0.275	0.245
30.0	0.35	----	-----	-----
45.0	0.34	----	-----	-----
60.0	0.33	----	-----	-----
120.0	0.32	----	-----	-----
150.0	----	0.10	-----	-----
Temp.	Room	Room	24-25°C.	35-36°C.
pH	9.8-10.1	9.0-10.7	9.2	9.2

(a) Reference 6, 520 mu, 5.0 cm.
(b) Reference 8, 540 mu.
(c) Reference 7, monomethyl ether of hydroquinone, 505 mu, 1.00 cm.

Table XIII

Effect of Time on Stability of the Color Complex at pH 8.0 [a]

2,4-Dichlorophenol	Absorbance [b]		
ug	15 min.	60 min.	120 min.
10	0.043	0.043	0.041
30	0.114	0.114	0.111
50	0.187	0.187	0.180
80	0.288	0.288	0.284
100	0.357	0.357	0.347

(a) Reference 4.

(b) 500 mu, 2.3 cm., room temperature.

was developed within 15 minutes and remained stable for at least two hours.

Discussion

The condensation of phenols with 4-aminoantipyrine with subsequent alkaline oxidation to a colored product appears to be two simple, straightforward chemical reactions. When these reactions are quantitated, however, several factors have to be considered. First, the pH value between 8.0 and 11.0 and type of buffer influence greatly the reactions. These two factors determine the absorbance value, wave length of maximum absorption, and the sensitivity with which a particular phenol will react. Also, the position, type, and number of substitutions on the phenol ring influence the sensitivity of the reaction. Substitution in the ortho and meta positions suppresses the reactions. Substitution in the para position by alkyl, aryl, ester, nitro, benzoyl, nitroso, amino, and aldehyde appears to block and/or inhibit the reactions, the degree of which is influenced by the pH value. Other factors of interferences, color complex stability, temperature, and order of reagent addition are eliminated or standardized by conventional analytical techniques.

Critical evaluation of the above factors relative to an analytical technique suggests that only an approximation can be made of the phenol content of a water or waste water. A "total phenol" content is not possible with the 4-aminoantipyrine method since many phenols are weakly reactive or do not react at all. Sufficient data are

available, however, to show that a closer approximation of the total phenol content can be obtained at a reaction pH of 8.0 than at 10.0. At pH 8.0, a greater number of the para- substituted phenols react.

Presumably, the reaction occurs as a coupling of the 4-aminoantipyrine at the para position of the phenol. This has never been directly established through a mechanism study. A para-condensation on the phenol ring is logical, however, since the OH substitution is ortho and para directing. Also, a para condensation is required for a quinoid structure to appear upon oxidation. Consequently, the reactivity of substituted phenols is determined by the combined influence of the groups that activate or deactivate the para-position. In general, the phenols that are substituted with ortho and para-directing groups appear to be more reactive than those with meta directing groups.

Summary

The quantitative determination of phenols in water and waste water is attempted by condensation with 4-aminoantipyrine and oxidation under alkaline conditions to a colored product. The major chemical factors that influence this reaction are pH value between 8.0 and 11.0, the type of aqueous buffer, and the type of substituted phenol. These three factors influence the analytical and molecular sensitivities as well as the wave length of maximum absorbance. A "total phenol" determination is not possible with the 4-aminoantipyrine technique as currently practiced.

Acknowledgement

Portions of this paper were supported by Research Grant WP 365 of the National Institutes of Health, Bethesda, Maryland

Literature Cited

1. Eisenstaedt (Emerson), E., Jour. Org. Chem. 3, 153 (1938).

2. Emerson, E., Jour. Org. Chem. 8, 417 (1943).

3. Emerson, E. and Kelly, K., Jour. Org. Chem., 13, 532 (1948).

4. Faust, S.D., and Aly, O.M., Jour. A.W.W.A., 54, 235 (1962).

5. Gottlieb, S. and Marsh, P.B., Anal. Chem., 18, 16 (1946).

6. Ettinger, M.B., Ruchhoft, C.C., and Lishka, R.J., Anal. Chem., 23, 1783 (1951).

7. LaCoste, R.J., Venable, S.H., and Stone, J.C., Anal. Chem., 31, 1246 (1959).

8. Ochynski, F.W., The Analyst, 85, 278 (1960).

9. Standard Methods for the Examination of Water and Waste Water. APHA, AWWA, WPCF, New York (11th ed., 1960).

10. Dannis, M., Sew. and Ind. Wastes, 23, 1516 (1951).

11. Mohler, E.F. Jr., and Jacob, L.N., Anal. Chem., 29, 1369 (1957).

12. Gordon, G.E., Anal. Chem., 32, 1325 (1960).

13. McFarlane, H.M., Proc. 10th Ontario Industrial Waste Conf. (1963).